Identification of Two Secreted Phospholipases A_2 in Human Epidermis

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Phospholipases A_2 are enzymes that catalyze the release of fatty acids from the *sn-2* position of phospholipids. Fatty acids have been suggested to play a key role in the barrier function of the epidermis. The aim of this study was to identify and characterize the type of secretory phospholipase A_2 expressed in human epidermis. We report the molecular cloning of two secretory phospholipase A_2 in the human epidermis. The first enzyme is identical to human pancreatic type IB phospholipase A_2. Western blots revealed a 14 kDa protein localized in the soluble fraction. The second phospholipase A_2 is identical to human synovial type IIA enzyme and is localized in the membrane fraction. By semiquantitative reverse transcription–polymerase chain reaction performed on horizontal sections of the epidermis, we found that the mRNAs of both phospholipases A_2 were expressed mainly in the basal layers of the epidermis. Our data thus provide evidence for the expression of two secretory phospholipases A_2 in human epidermis. The different localization of these two secretory proteins strongly suggests that each enzyme might have a specific role in skin physiology and probably in the barrier function. Taken together, these data validate the reverse transcription–polymerase chain reaction technique performed on thin sections as a first approach to detect gene expression in different layers of the epidermis. ***Key words: cloning/human epidermis/localization/low molecular weight phospholipase A_2. J Invest Dermatol 114:960–966, 2000***

Phospholipases A_2 (PLA_2) are enzymes that catalyze the hydrolysis of the *sn-2* fatty acyl ester bond of phospholipids to generate free fatty acid and lysophospholipid. Mammalian PLA_2 are subdivided into two major families: low molecular mass secretory enzymes ($sPLA_2$) consisting of four types (I, II, V, and X), and high molecular mass cytosolic PLA_2 existing as two types (IV or $cPLA_2$ and VI or $iPLA_2$) (Dennis, 1997; Balsinde *et al*, 1999).

Type I PLA_2 is found mainly in pancreatic secretion, lung (Pruzanski and Vadas, 1990) and vascular smooth muscle cells (Nakajima *et al*, 1992). Type II PLA_2 was identified in inflammatory synovial fluid, platelets, and other tissues such as digestive tract and prostate (Nevalainen and Haapanen, 1993). The last identified enzymes (types V and X) are present in human testis and thymus, respectively (Cupillard *et al*, 1997). Type IV PLA_2 displays ubiquitous distribution (Dennis, 1997) whereas type VI PLA_2, was identified in the macrophage-like $P388D_1$ cell line, as well as Chinese hamster ovary cells (Ackermann *et al*, 1994; Tang *et al*, 1997).

Both membrane bound or extracellular $sPLA_2$ are activated by millimolar Ca^{2+} concentrations. They display a broad substrate specificity and contain several disulfide bonds, which enhance their stability in extracellular media. In contrast, type IV cytosolic PLA_2 ($cPLA_2$) is specific for phospholipids bearing arachidonic acid at *sn-2* position and cannot be inhibited by reducing agents (Dennis *et al*, 1991). Type IV $cPLA_2$ is translocated to the membrane in the presence of submicromolar Ca^{2+} concentrations (Kramer *et al*, 1991) and is activated by phosphorylation (de Carvalho *et al*, 1996), whereas type VI PLA_2 is calcium-independent and probably activated by adenosine triphosphate (Lio and Dennis, 1998).

PLA_2 are involved in many physiologic and pathologic conditions (Balsinde *et al*, 1999). Type IV PLA_2 hydrolyzes *sn-2*-acyl ester bond to generate arachidonic acid which is an eicosanoid precursor (Murakami *et al*, 1997). $sPLA_2$ are involved in ischemia (Rordorf *et al*, 1991), catecholamine secretion (Matsuzawa *et al*, 1996), lysophosphatidic acid production (Howe and Marshall, 1993), and anti-coagulation (Kini and Evans, 1987). Their synthesis is increased in various cells during inflammatory processes, resulting in their accumulation in synovial fluid or in plasma of patients suffering from septic shock (Vadas *et al*, 1993).

Several authors detected PLA_2 activity in epidermis (Freinkel and Traczyk, 1985; Bergers *et al*, 1986). Both $cPLA_2$ and $sPLA_2$ might be involved in phospholipid remodeling occurring during epidermal differentiation (Elias *et al*, 1981; Cox and Squier, 1986) and $sPLA_2$ probably contributes to the structuration of lamellar bodies (Elias, 1996). Their activity is enhanced during skin ultraviolet injury (Kang-Rotondo *et al*, 1993), and in psoriasis (Forster *et al*, 1985). Besides, type II enzyme and not type IV is overexpressed in psoriasis (Andersen *et al*, 1994). We recently identified type I PLA_2 in human epidermis. This enzyme is localized at the stratum corneum/stratum granulosum junction (Mazereeuw-Hautier *et al*, 2000). Mouse epidermis seems to express several PLA_2 such as type I and II (Li-Stiles *et al*, 1998).

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Abbreviation: PLA_2, phospholipases A_2.

These data suggest the presence of several forms of PLA_2 in human epidermis. Several lines of evidence suggested that secretory enzymes play a major part in barrier function (Mao-Qiang *et al*, 1995, 1996) or in pathologies of the skin (Andersen *et al*, 1994; Li-Stiles *et al*, 1997). Therefore, the focus of our study was to identify secretory PLA_2 in human epidermis. Herein, we report that human epidermis expresses two secretory PLA_2. By molecular cloning, we showed that the first is identical to pancreatic enzyme and belongs to type IB. The second has the same amino acid sequence as type IIB human synovial PLA_2. Type I PLA_2 is expressed in keratinocytes. Type II enzyme is mostly expressed in keratinocytes and weakly in dendritic cells. We found these two enzymes in normal healthy epidermis, suggesting a role for both enzymes in the differentiation and probably in the barrier function of the epidermis.

MATERIALS AND METHODS

Cell culture media and reagents TRIzol were from Life Technologies (Cergy-Pontoise, France). Reverse transcription system, Taq DNA polymerase, pGEM-T vector, Wizard Miniprep DNA purification system, and restriction enzyme were purchased from Promega. (Charbonnieres, France). Oligonucleotides were obtained from Isoprim (Toulouse, France). ''Protease inhibitor cocktail for mammalian cells extracts'' and RPMI were from Sigma (Saint-Quentin-Fallavier, France) and Hybond C Extra from Amersham (Saclay, France). Type IV PLA_2 antibody was obtained from TEBU (Le Perray en Yvelines, France), type I PLA_2 antibody from Upstate Biotechnology Inc., Euromedex (Souffelweyersheim, France), type II PLA_2 antibody from Boehringer Mannheim, Roche Molecular Biochemicals (Meylan, France), and LumiGlo kit from New England Biolabs (Ozyme, Saint-Quentin-en-Yvelines, France). Sequencing was performed by Genome Express S.A. (Grenoble, France). LSAB Kit and anti-cytokeratin 1 and 10 antibodies were from DAKO (Trappes, France). Reconstructed human epidermis was purchased from Skinethic Laboratories (Nice, France).

Human skin specimen Healthy abdominal and mammary skins were obtained from plastic surgery from 18 to 50 y old women.

Sections of human epidermis Fragments of human skin (5 mm^2) were rapidly washed twice with phosphate-buffered saline (PBS), embedded in tissue tek and sectioned (10 μm thickness) with a cryomicrotome (Toulouse, Leica). The cutting plan was longitudinal so frozen sections were parallel to the epidermis. Each section was cut into two pieces. The first half was collected on Superfrost Plus precoated microscope slides and analyzed by immunohistochemistry, the second half was collected in 1 ml of TRIzol reagent and submitted to total RNA extraction.

Cell culture Human epidermal keratinocytes were obtained by treating skin with 0.25% trypsin overnight at 4°C (Bata-Csorgo *et al*, 1995). Keratinocytes were grown in keratinocyte serum-free medium (Keratinocyte-SFM) supplemented with bovine pituitary extract (30 μg per ml), epidermal growth factor (0.1 ng per ml) and gentamycin (80 μg per ml). When they have reached 70% confluency, keratinocytes were trypsinized (0.05% trypsin, 0.02% ethylenediamine tetraacetic acid) collected and RNA extracted. For dendritic cells preparations, the fat was stripped from the skin which was washed 10-fold for 10 min in PBS containing ciflox (4.5 μg per ml) and gentamycin (170 μg per ml). Skin was then cut into 25 mm^2 fragments and incubated with RPMI containing 10% decomplemented bovine serum albumin at 37°C. After 3 d of incubation, dendritic cells from the explants were collected with a pipette, centrifugates at $100 \times g$ centrifugation and RNAs were extracted.

RNA extraction Immediately after surgery, skins were chilled on ice. Within 15 min, the epidermis and superficial parts of the dermis were recovered by firm scrapping with a scalpel (Hansson *et al*, 1994), homogenized with a Dounce homogenizer in TRIzol reagent and total RNA extracted as suggested by the supplier. Reconstructed epidermis was homogenized and RNA extracted according to the same protocol. RNA from scrapped keratinocytes, dendritic cells and frozen skin sections were extracted directly, without Dounce homogenization.

cDNA synthesis First-strand cDNA was generated from 1 μg total RNA using oligo(dT) priming and avian myeloblastoma reverse transcriptase (Reverse transcription system) at 42°C for 45 min.

Primer design We designed our own specific primers for each type of secreted PLA_2. These primers were chosen at both extremities of each human PLA_2 sequences described in GenBank/EMBL databases: accession numbers M22970 for type I PLA_2 and, respectively, NM 002023.2, NM 000929.1 and NM 003561 for types II, V, and X PLA_2. Moreover, a restriction enzyme site was included in the sequence of some primers to introduce polymerase chain reaction (PCR) products in the cloning vector. The Tm of each primer was calculated with the oligocalculator program (EMBL). The primers used for type I PLA_2 were 5-GCTTC-TCTAGAAATGGCCGTGTGGCAGTTCCGCAAAATG-3 (PLF1, forward, Tm = 69°C, the *Xba*I restriction-site is underlined) and 5-GTGACAGTCGACTGAGAGGTGATATTCAACTCTGAC-3 (PLR1, reverse, Tm = 66°C, *Sal*I restriction-site underlined). The primers used for type II PLA_2 were 5-ATCCGGAGATCTCAAGAGCTCTTACCATG-3 (PLF2, forward, Tm = 62°C, *Xho*II restriction-site underlined) and 5-AAAAGGTCTAGAAGGGAAGAGGGGACTCA-3 (PLR2, reverse, Tm = 62°C, *Xba*I restriction-site underlined). The primers for type V PLA_2 were 5′-AGTTATGGCTTCTACGGCTGCCACTG-3′ (PLF5 forward, Tm = 61°C, chosen in the calcium binding loop) and 5-TACGAGCTTCCGGTCACAGGCAC-3 (PLR5, reverse, Tm = 61°C) and for type X, 5-GGGATCCTGGAACTGGCAGGAAC-3 (PLF10, forward, Tm = 61°C) and 5-TCAGTCACACTTGGGCGAGTCCG-3 (PLR10, reverse, Tm = 61°C). Primers for human β-actin were 5-CTGGAACGGTGAAGGTGACAC-3 (ACTF, forward, Tm = 56°C) and 5-GGTCTCAAGTCAGTGTACAGG-3 (ACTR, reverse, Tm = 54°C).

PCR PCR amplifications were performed using a DNA thermal cycler (Crocodile III, Appligen Oncor, Illkirch) in a 50 μl mixture. Following buffer conditions were used: 10 mM Tris–HCl, pH 9, 3.5 mM magnesium chloride, 50 mM potassium chloride, 0.1% Triton X-100, 200 μM of each dATP, dGTP, dCTP, dTTP, 2.5 units of Taq DNA polymerase (Promega) and 50 pmol of each primer. After an initial incubation of 1 min at 94°C, amplification was achieved for 30 cycles as followed: 1 min at 94°C, 1.5 min at annealing temperature, and 1 min at 74°C. The annealing temperatures were 62°C (amplifications of PLA_2 types I, V, and X), 55°C for PLA_2 type II with the primers PLF2 and PLR2, and 56°C for β-actin.

Cloning and sequencing PCR products PCR products were purified from agarose gel and cloned into pGEM-T easy vector according to the manufacturer protocol. Plasmid DNA was extracted from an overnight culture of bacteria with the Wizard Miniprep DNA purification system. The insert was excised by *Eco*RI digestion and clones with the expected size insert were sequenced.

Epidermis homogenate preparation After surgery, skin pieces were washed twice in PBS. Epidermis was separated from the dermis by heating in a 60°C water bath for 5 min followed by quick chilling in ice. Epidermis was then removed with forceps and cut into small pieces. They were then disrupted by Dounce homogenization in a buffer (Tris–HCl 62.5 mM, pH 6.8, sodium dodecyl sulfate 1.5%) containing protease inhibitors (protease inhibitor cocktail for mammalian cells extracts) at the concentration of 1 μl for 20 mg of epidermis. After $1000 \times g$ centrifugation to remove nuclei and cellular debris, the supernatant was centrifuged at $100{,}000 \times g$ for 60 min at 4°C to obtain cytosolic and membrane preparations. Post-nuclear supernatant, cytosolic and membrane fractions were subjected to protein quantitation and immunoblotting assays.

Immunoblotting Eighty micrograms of proteins from postnuclear supernatant or 40 μg from cytosolic and membrane fractions were subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis on a 15% (wt/vol) polyacrylamide gel under reducing conditions (Laemmli, 1970) and transferred to nitrocellulose membranes (Hybond C extra, Amersham, Saclay). Immunoreactive proteins were detected with 0.2 μg per ml of anti-human type IV PLA_2 monoclonal antibody, 2 μg per ml of the anti-porcine type I PLA_2 polyclonal antibody or 5 μg per ml of anti-human type II PLA_2 monoclonal antibody. Antibodies were diluted in TBS–tween buffer (10 mM Tris, pH 8, 150 mM NaCl, Tween-20 0.05%) supplemented with 3% bovine serum albumin and the incubation was performed for 1 h at room temperature. PLA_2 were then detected by ECL with LumiGlo kit.

Immunohistochemistry After acetone fixation, human skin sections were incubated with a monoclonal antibody directed against cytokeratins 1 and 10 that specifically labels the suprabasal layers of the epidermis. The following protocol was applied. Sections were rehydrated in PBS for 10 min, and nonspecific binding of the antibody was blocked by incubation

in PBS supplemented with 3% bovine serum albumin for 15 min. The antibody at 1/100 dilution in PBS was incubated for 1 h at room temperature. Keratins were then detected using the LSAB kit. To visualize all the layers of the epidermis easily, slides were labeled with Harris's hematoxylin for 30 s.

Miscellaneous Proteins were quantitated by the method of Lowry in the presence of sodium dodecyl sulfate (0.07%, wt/vol) with bovine serum albumin as a standard (Lowry *et al*, 1951).

RESULTS

Identification of PLA_2 mRNA in human epidermis In order to recover epidermis, skins were soaked in RNAse free water at 65°C for 15 min followed by rapid chilling on RNAse free ice. Under these conditions, the epidermis dissociates from the dermis easily. With this procedure, RNA was completely degraded, so we decided to scrape the epidermis with a scalpel, which led to recovery of intact RNA. In order to determine which $sPLA_2$ was expressed in human epidermis, we performed reverse transcription–PCR with primers specific for the described mammalian enzymes. We tested primers for types I, II, V, and X PLA_2. Using the primers PLF5–PLR5 and PLF10–PLR10 we failed to detect any expression of type V and X transcripts in the epidermis. We detected the type I PLA_2 (**Fig 1*a***) and more surprisingly the type II PLA_2 (**Fig 1*b***) in all noninflammatory samples of epidermis. The negative control consists of the PCR reaction mixture without template.

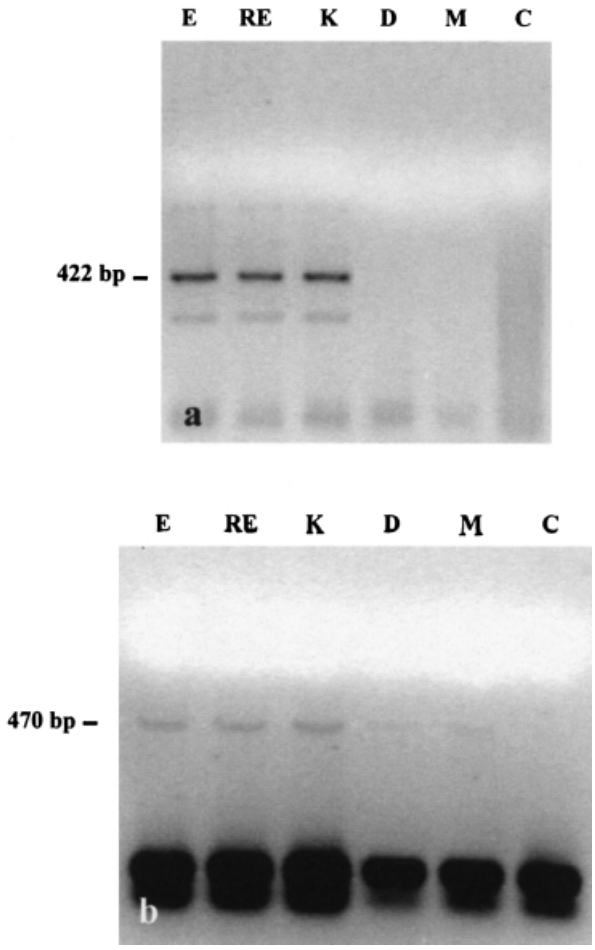

Figure 1. PCR amplification and localization of type I and II $sPLA_2$ in human epidermis. Reverse transcription–PCR performed with specific primers were conducted as described in *Materials and Methods* with the two couples of primers PLF1–PLR1 and PLF2–PLR2. The expected bands were 422 bp for type I PLA_2 (*a*) and 470 bp for type II PLA_2 (*b*). E, epidermis; RE, reconstructed epidermis; K, primary culture of keratinocytes; M, melanocytes; D, dendritic cells; C, control without RNA.

Localization of both transcripts in the epidermis In order to know which cells express type I and II PLA_2, we performed reverse transcription–PCR with RNA extracted from the whole epidermis or reconstructed epidermis described to be histologically similar to the natural epidermis and displaying correct barrier function (Rosdy and Clauss, 1990; Rosdy *et al*, 1996). Moreover, we performed reverse transcription–PCR on RNA extracted from different epidermic cell types: keratinocytes, melanocytes, or dendritic cells. Keratinocytes were grown in conditioned medium made to reduce fibroblast contamination. Reverse transcription–PCR was performed on melanocytes RNA extracted from secondary culture. This cell culture contained only melanocytes similar to normal adult human melanocytes *in vivo* (Donatien *et al*, 1993). We also performed reverse transcription–PCR on dendritic cells obtained from skin explants. This cell preparation contains dendritic cells derived from the epidermis (Langerhans cells) and dermic dendritic cells. Our results (**Fig 1**) showed that type I PLA_2 transcripts were only observed in epidermis, reconstructed epidermis, and cultured keratinocytes (**Fig 1*a***), confirming the presence of this enzyme in normal epidermis, and discriminates against a contamination with dermic cells. As shown in **Fig 1(*b*)**, reverse transcription–PCR performed with type II PLA_2 primers gave identical results. A weak band, however, was found in melanocytes and dendritic cells. In the case of dendritic cells, type II PLA_2 could be expressed by Langerhans cells as well as other dendritics cells of the dermis. The data presented in **Fig 1**, however, indicate that type II PLA_2 is expressed mainly in keratinocytes. All the PCR products were cloned and sequenced to make sure they matched with type I and II PLA_2.

Molecular cloning of the epidermal type I and II PLA_2 We cloned products corresponding to the predicted sizes (422 bp for type I PLA_2 and 470 bp for type II) (**Fig 2**). Comparison of amino acid sequences with other mammary secretory PLA_2 was performed with the ClustalW program. The epidermal specific type I PLA_2 has an amino acid sequence identical to other human type I PLA_2 such as the lung form (Seilhamer *et al*, 1986) and it bears the signal peptide characteristic of eucaryotic secretory proteins (von Heijne, 1986). Epidermal type II PLA_2 is as well identical to other type II PLA_2 such as the synovial form (Seilhamer *et al*, 1989).

Immunologic detection of epidermal PLA_2 To characterize better the secreted PLA_2 expressed in epidermis, we performed Western blots with antibodies against type I and II PLA_2. We detected a 14 kDa band (i.e., the size expected), with anti-porcine pancreatic antibody (**Fig 3*a***). As shown in **Fig 4(*b*)**, with anti-human type II PLA_2 antibody, we again detected immunoreactive labeling at 14 kDa, confirming the presence of type II PLA_2 in epidermis. Control incubations without the primary antibodies did not show any staining (not shown). As depicted in **Fig 3(*c*, *d*)**, type II PLA_2 was found only in the membrane fraction and the type I PLA_2 in the soluble fraction. A Western blot using an antibody against cytosolic PLA_2 (type IV PLA_2) was performed as a control (**Fig 3*e***). This enzyme was detected as a single 100–110 kDa band in the soluble fraction as previously observed (Li-Stiles *et al*, 1998; Furstenberger *et al*, 1997).

Localization of both type I and II PLA_2 in the epidermis In order to precise, *in situ*, the cellular origin of the PLA_2 in epidermis, we developed an original and sensitive method to detect specific type I and II RNA. This method, based on reverse transcription–PCR performed on skin sections, happened to be more sensitive and rapid than *in situ* hybridization. The skin was cut into sections that paralleled to the epidermis. Each section was labeled with hematoxylin that stained nuclei in blue. Epidermis suprabasal layers keratins K1–K10 were codetected by immunohistochemistry and appeared in brown with our detection method. The first sections (**Figs 4*a*, *b***) contained cells with hematoxylin labeled nuclei and

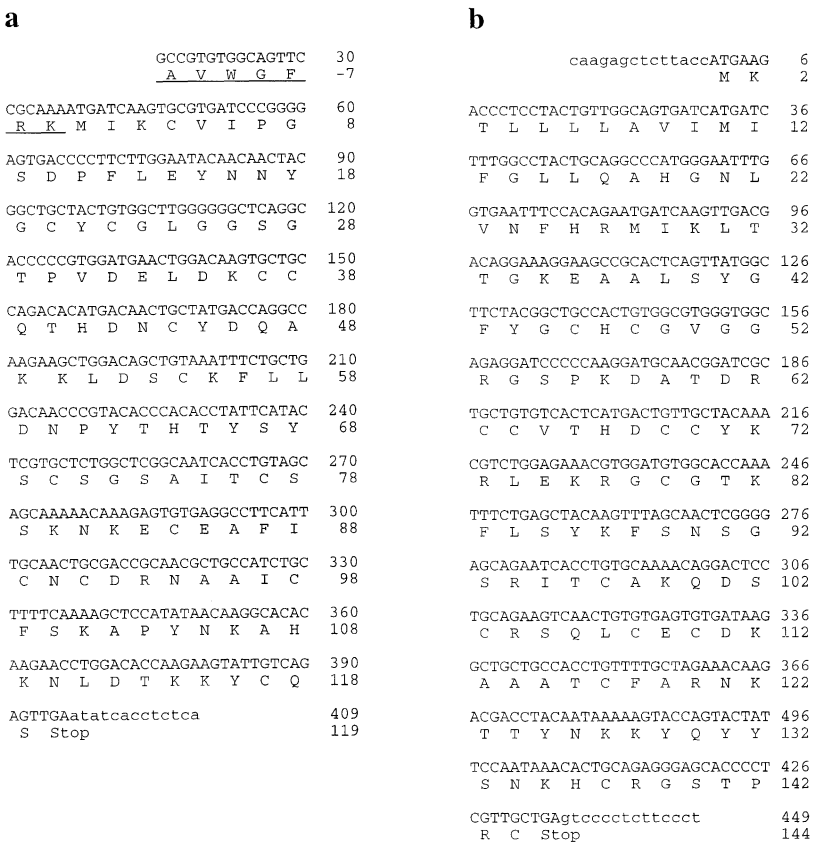

Figure 2. Nucleotide and predicted amino acid sequences of human epidermis type I and type II PLA_2. (*a*) Type I PLA_2; (*b*) type II PLA_2. The partial sequence of the signal peptide is underlined.

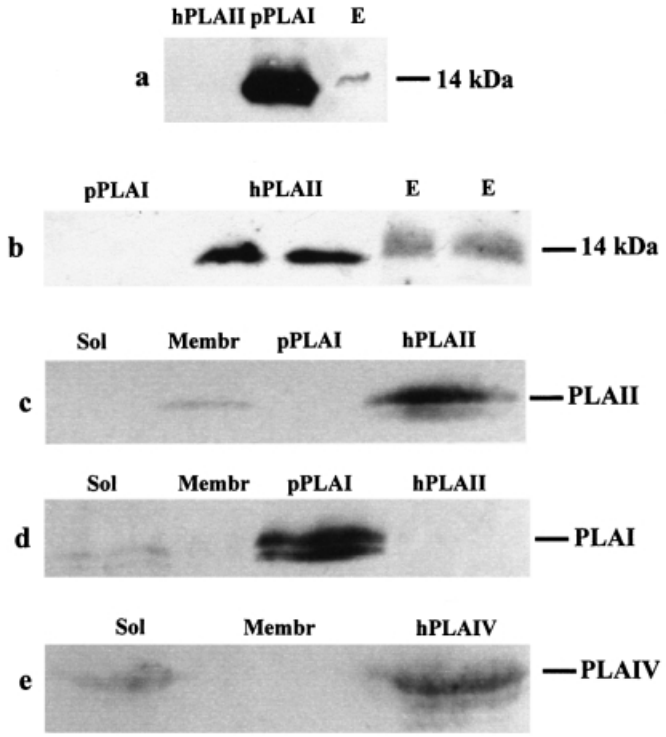

Figure 3. Immunoblot and localization by subcellular fractionation of type I and II PLA_2 in the human epidermis. (*a, d*) Western blots performed with anti-porcine type I PLA_2 antibody; (*b, c*) western blots with the antibody anti-type II PLA_2. pPLAI, porcine recombinant type I PLA_2 (Boehringer Mannheim). $hPLA_2$ II, human recombinant type II PLA_2; (*e*) western blot performed with anti-human type IV PLA_2 antibody. E, epidermis homogenate, Sol, soluble fraction, Membr, membrane fraction, hPLAIV, control with 10 μg total homogenate α2AF2 preadipocytes (Pages *et al*, 1999).

with a brown labeled cytosol typical of epidermis corneus and suprabasal layers. Sections 3 and 4 show deeper cuts, i.e., more basal layers. The dermis invaginations appeared as nonlabeled circles containing sparse nuclei (**Fig 4*c***). At the periphery of the invaginations, we could visualize the basal layer of the epidermis that was not immunolabeled in brown with the anti-keratin 1 and 10 antibody and appeared as a thin layer of nuclei. In the fourth section (**Fig 4*d***) more dermic invaginations were present. In the fifth and last section (**Fig 4*e***), dermis was the main tissue and covered very large areas. We performed semiquantitative reverse transcription–PCR on each section. Control amplification with β-actin specific primers showed nearly the same intensity in sections 2–4. In the first section we did not find any β-actin expression probably because of RNA degradation in the highest layer of the epidermis. Type I and II PLA_2 were largely expressed in sections 2–4 confirming their ubiquitous distribution in the keratinocytes of the epidermis (**Fig 5**). Expression peaked in the fourth section which corresponds to the more basal layers of the epidermis. In the fifth section, when the dermis predominates, and actin amplification were maximal, we did not amplify type I PLA_2 RNA and only a weak expression of type II PLA_2 could be detected. No template RNA was included as the negative control (data not shown).

DISCUSSION

The purpose of this study was to identify positively the type of secretory PLA_2 expressed in human epidermis. Previous reports suggested the presence of several PLA_2 in epidermis. Data based on PLA_2 activity measurements suggested the existence of a secretory enzyme (Forster *et al*, 1983; Bergers *et al*, 1986). Bastian *et al* (1996), identified type II PLA_2 in keratinocytes stimulated by interleukin-1α and tumor necrosis factor-α, and an increase in gene expression was reported in psoriasis (Andersen *et al*, 1994). Recently, Li-Stiles *et al* (1998) found two secretory PLA_2 in mouse skin. Moreover, recent investigations in our laboratory provided strong evidence for the existence of a group I PLA_2 in epidermis (Mazereeuw-Hautier *et al*, 2000). No direct characterization of these enzymes in epidermis,

however, had yet been achieved. In this study, we report the molecular cloning of two secretory PLA_2 in human epidermis (**Fig 2**).

The first enzyme is identical to pancreatic or lung PLA_2 and belongs to type IB (Dennis, 1997). The mRNA of this enzyme is mainly localized in basal layers of the epidermis (**Fig 5**) and the

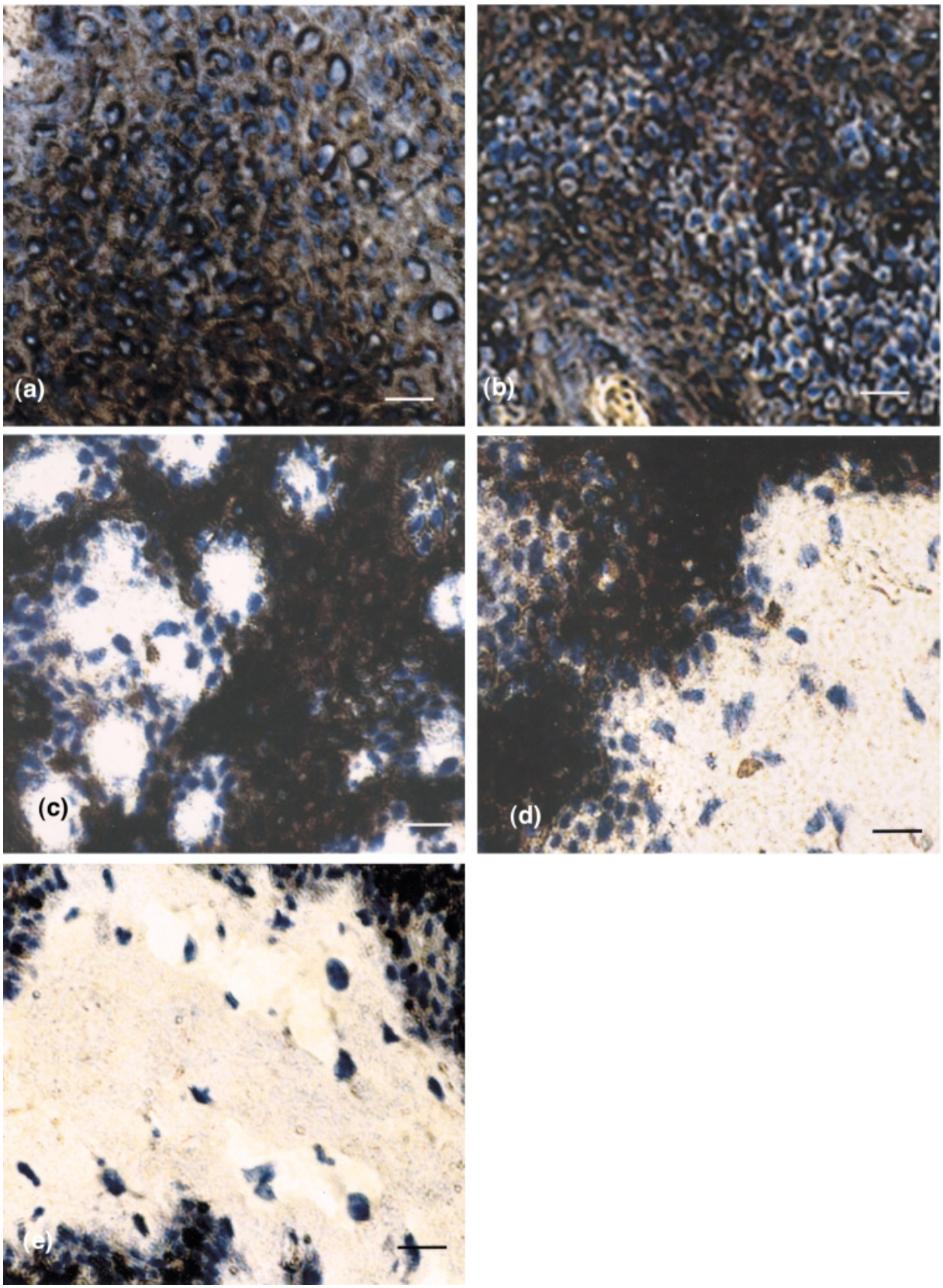

Figure 4. Immunohistochemistry of epidermis sections labeled with anti-keratin 1 and 10 antibody and hematoxylin. Five horizontal slides from the outside (*a*) to the inside (*e*) of the same skin. The immunolabeling with anti-keratin 1 and 10 antibody appeared in brown, nuclei were blue, and cytosol of dermis cells were white. Numbers correspond to slides made from the outside to the inside of the skin. *Scale bar*: 10 μm.

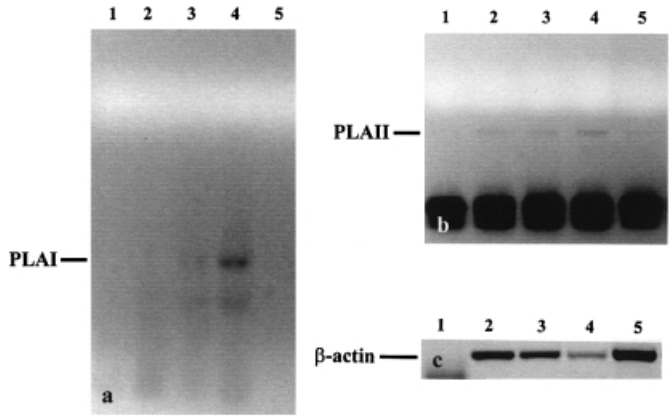

Figure 5. Reverse transcription–PCR amplification of type I and II PLA_2 and β-actin in human epidermis horizontal sections. (*a*) Type I PLA_2; (*b*) type II PLA_2; (*c*) β-actin. Reverse transcription–PCR were performed with three couples of primers: PLF1–PLR1, PLF2–PLR2, and ACTF–ACTR with 250 ng of total ARN from each section. The predicted size with β-actin primers is 420 bp. The numbers correspond to horizontal sections made from the outside to the inside of the skin.

protein is present in soluble fraction confirming its secretory character (**Fig 3**). Amino acid sequence of the epidermis type I PLA_2, as the pancreatic enzyme, bears a serine protease cleavable signal peptide. This suggests that epidermis type I PLA_2 could behave as the pancreatic enzyme. This skin PLA_2 would be synthesized as an inactive proenzyme that would then be cleaved by serine protease(s) to release an active mature enzyme. Interestingly, several authors characterized serine proteases such as trypsin-like (Cui *et al*, 1997) and chymotrypsin (Hansson *et al*, 1994) proteins in the skin. These serine proteases seem to localize in the stratum corneum for the trypsin (Cui *et al*, 1997; Redoules *et al*, 1998) and mainly in the stratum granulosum for chymotrypsin (Sondell *et al*, 1994). In a previous study (Mazereeuw-Hautier *et al*, 2000), type I PLA_2 was localized at the junction between the stratum granulosum and the stratum corneum. Taken together, these data support the idea that type I PLA_2 could be probably secreted by lamellar bodies in the stratum granulosum–stratum corneum junction where it would be cleaved by serine proteases. Consequently, this PLA_2 would be activated and might hydrolyze phospholipids on the lamellar bodies to generate fatty acids that participate to the skin barrier function (Mao-Qiang *et al*, 1995).

Figure 5 shows that PLA_2 mRNA expression decreases with the epidermal differentiation. This could be easily explained by the fact that epidermal differentiation is concomitant with progressive degradation of nuclei with subsequent decrease in RNA transcripts. So, it is legitimate to imagine that during epidermal differentiation PLA_2 mRNA would be less and less transcribed leading to a progressive decrease of detection by semiquantitative reverse transcription–PCR.

The second PLA_2 cloned in human epidermis is a group IIA enzyme (**Fig 2**) and its amino acid sequence is identical to the human synovial protein. This protein is localized in the membrane fraction as it has already been observed for the same enzyme from rat (Ono *et al*, 1988). Group II is believed to be an inflammatory enzyme expressed in pathologic synovial fluid (Pruzanski *et al*, 1991), septic shock (Vadas *et al*, 1988), and cancer (Murata *et al*, 1993). This protein is implicated in psoriasis, the mRNA not being detected by northern blot techniques in healthy tissue (Andersen *et al*, 1994). By reverse transcription–PCR, a more sensitive method (**Fig 1**), and by western blotting (**Fig 3**), we detected the presence of group II PLA_2 in healthy epidermis showing that this enzyme has a role under nonpathologic conditions.

It may be quite surprising to find two secretory enzymes, type I and II PLA_2, in the same tissue. This situation, however, has already been observed in other tissues such as in duodenum, jejunum, or lung (Nevalainen and Haapanen, 1993). If type I and II enzymes share the same molecular weight, calcium dependency, disulfide bounds, and active site, some substantial differences are also observed regarding to the amino acid sequence (36% homology) and substrate specificity (phosphatidylglycerol specificity for type I PLA_2 (Tojo *et al*, 1988) compared with phosphatidic acid or phosphatidylserine specificity for type II PLA_2 (Snitko *et al*, 1997). Because of the localization of phosphatidylserines in the inner leaflet of the membranes, type II PLA_2 is more active on membranes with altered phospholipid asymmetry. This perturbation occurs when cells are activated (Fourcade *et al*, 1995) or during apoptotic events (Fadok *et al*, 1998; Huppertz *et al*, 1999). Therefore, differentiating keratinocytes have been reported to share some features with apoptotic cells such as DNA fragmentation or caspase activation (Weil *et al*, 1999). Because type II PLA_2 hydrolyzes phospholipids in disturbed membranes, this enzyme might play an important part during epidermal differentiation.

We have noticed the presence of both secretory type I and II PLA_2 in reconstructed epidermis at all differentiation stages: at the first stage corresponding to the 15th day of culture when the reconstructed epidermis structurally appeared as normal epidermis, and at the 20th and 25th days when the stratum corneum became abnormally thicker (data not shown). This indicates that reconstructed epidermis is a good model to study the pathophysiologic implication of the two PLA_2 as well as for pharmacologic studies.

In this study, we show that PLA_2 are expressed mainly in keratinocytes (**Fig 1**). We then wanted to achieve *in situ* localization of the RNA of each enzyme. The commonly used method is *in situ* hybridization but it is efficient for sufficiently expressed mRNA. Northern blots performed with epidermis RNA did not allow us to detect any PLA_2 transcript (data not shown) confirming previous data (Andersen *et al*, 1994) and suggesting the level of expression of the mRNA encoding these enzymes is very low. Under these conditions, we could not use the *in situ* hybridization techniques, which are not sensitive enough. Therefore, we chose to develop another detection method based on reverse transcription–PCR. We thus decided to cut the epidermis into sections which paralleled the dermis and we determined the expression level of type I and II PLA_2 mRNA in each section by semiquantitative reverse transcription–PCR. Even if the horizontal sectioning does not allow clear identification of the RNA producing cells and is only informative in term of localization in the different layers of the epidermis, our method has the advantage of giving a quick answer, in contrast to *in situ* hybridization that requires several weeks of exposure when using sensitive radiolabeled probes. This method is easy to perform and does not use radioactive material which identifies horizontal sectioning coupled to reverse transcription–PCR as a convenient method for the detection of mRNA in the epidermis as a first localization approach. It is also an alternative method when *in situ* hybridization failed because of its low sensitivity.

In pathologic studies it would be interesting to know whether type I and II PLA_2 expression is altered in dermatoses in which the barrier function is impaired. Several reports described modifications of secretory PLA_2 activity in psoriasis (Forster *et al*, 1985). All those studies did not identify the specific forms of PLA_2 implicated. Andersen *et al* (1994) showed an increase in the expression level of type II PLA_2 in psoriasis but did not look for type I enzyme. It will be interesting to investigate the expression level of these enzymes and particularly type I PLA_2 with immunohistochemistry and horizontal sectioning in psoriasis.

The specific and different localization of these secretory PLA_2 strongly suggest that they might have a specific role in skin physiology.

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